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(54) Title: DIRECT SPRAY-DRIED DRUG/LIPID POWDER COMPOSITION (57) Abstract The present invention discloses a method for direct spray-drying a solution of lipids and water soluble drug to generate a bulk powder as an alternative to the drying of preformed liposomes. In the present method the lipids are dissolved in a solvent and the water-soluble drug is dissolved in aqueous solvent. The two solutions are combined to form a precipitate-free feed solution which is then spray-dried to generate the bulk powder. Upon rehydration the powder spontaneously forms liposomes having a high drug encapsulation efficiency of approximately 70 %. The direct spray-dried powder is particularly useful for drug administration by inhalation.		

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DIRECT SPRAY-DRIED DRUG/LIPID POWDER COMPOSITION**Field of the Invention**

- 5 The present invention relates to drug delivery by inhalation, and, in particular, to a method of preparing direct spray-dried drug/lipid powders containing a metered drug dose for use via inhalation.

References

- Maniatis, T., et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Lab., Cold Spring Harbor, NY (1982).
- Bartlett, G.R., J. Biol. Chem. 234:455 (1959).
- 15 Fisher, K., as referenced in Analytical Chemistry, 14th Ed., Editor - Gary D. Christian.
- McGurk, J. G., et al., UK Patent Application GB 2 145 107 A (1985).
- Leigh, S., EPO Patent Application 0 158 441 (1986).
- 20 Carman-Meakin, B., et al., PCT Application WO 86/01714 (1986).
- McGurk, J. G., et al., UK Patent Application GB 2,170,815 (1988).
- Leigh, S., PCT WO/87/07502 (1987).
- 25 Radhakrishnan, R., et al., U. S. Patent No. 4,895,719 (1990).

Background

Inhalation provides an effective means for delivering a variety of drugs, including nasal decongestants, drugs useful in the treatment of asthma and other bronchial and pulmonary conditions. One advantage of inhalation in treating nasal, bronchial, and pulmonary conditions is the ability to deliver the drug directly to the site of drug action. A related advantage is the rapid onset of the therapeutic effect, compared with other routes of administration such as intramuscular and oral routes. For drugs which are susceptible to breakdown in the gastrointestinal tract, or which otherwise cannot be administered orally, inhalation may be preferred for a variety of reasons over intravenous or intramuscular injection. Other drugs, such as nitroglycerin, whose primary drug action is systemic, can also be delivered efficiently by inhalation.

Several methods for delivering drugs via inhalation are known. In one, the drug is dissolved in a suitable solvent which can be aerosolized to form a small-particle mist. The drug solution may be aerosolized by pneumatic or ultrasonic nebulizer or, more conveniently, by means of a self-contained nebulizer containing a pressurized, fluorocarbon propellant. Inhalation of the aerosol mist, i.e., drawing the mist from the mouth or nose into the respiratory tract, acts to deposit the drug-containing aerosol particles on various sites of the respiratory tract, including the upper nasopharyngeal region, the tracheobronchial region, and the pulmonary region. In the latter region, the drug has the opportunity for rapid absorption into the bloodstream for systemic action.

Also well known in the prior art are inhalation systems in which a drug is administered in particulate form, either as a dry powder or as a micronized suspension in a suitable carrier solvent system. Typically the drug is a water-soluble compound which is suspended in micronized form in a fluorocarbon-type propellant

solvent. Following aerosolization, most of the propellant solvent is lost through flash evaporation and replaced by moisture in the respiratory tract, leading to the deposition of hydrated micronized particles.

- 5 Both types of inhalation systems mentioned above are based on delivery of the drug in a free form to sites in the respiratory tract. As such, the drug is rapidly utilized and, in the case of pulmonary deposition, taken up systemically at the site of deposition. Because of
10 this rapid drug uptake and utilization, the drug effect may be relatively short-lived, requiring frequent dosing. A related problem is the limited amount of drug that can be administered safely at each dosing, particularly where the drug has unwanted systemic side effects. This problem is illustrated by a number of β_2 -adrenergic agonist
15 type bronchodilators which also produce marked tachycardia. Even at relatively low doses of these drugs, the stimulatory effect of the drug on the heart and other side effects, such as dizziness and insomnia, are a nuisance to the patient. Additionally, micronized particles may irritate the respiratory tract.

- More recently, liposome inhalation systems for administering a drug to the respiratory tract in liposome-entrapped form have been proposed. UK Patent Application GB 2,145,107A describes an aerosol device which
25 brings an aqueous solution and organic-solvent phase solution, in which the lipid components are dissolved, together under pressure, and passes the mixture through a nozzle to form aerosolized liposomes. EPO Patent application 0.158,441 discloses liposome formation, in
30 aerosol form, from a water/solubilized lipid/ethanol mixture. In PCT Application WO 86/01714, it is proposed to spray lipid droplets in a volatile liquid carrier (which is a solvent for the phospholipids), with liposome
35 formation occurring upon contact of the droplets with a moist aqueous surface. UK Patent Application GB 2,170,815 describes a system in which an aqueous solution

is emulsified in a propellant solvent in which lipid has been dissolved, then sprayed through an atomizing nozzle to form lipid-coated droplets which can form liposomes upon contact with a moist surface. PCT WO/87/07502 describes a sprayable composition consisting of a propellant solvent in which at least one membrane lipid is partially dissolved or dispersed and a biologically active compound which is insoluble in the solvent. All of these approaches are characterized by a multiphase solvent system in the atomizer wherein one phase contains dissolved lipid components. None of the prior art references teach bulk direct spray-drying of a drug/lipid solution to generate a drug/lipid powder effective to form liposomes with a high drug encapsulation efficiency upon rehydration of the dried powder particles.

Further, the concentration and size of the liposomes formed by the prior art methods, and the percentage of drug entrapment in the liposomes, will vary from one dose delivery to another, depending upon temperature and humidity conditions, the extent of solvent mixing, and the total and relative amounts of solvent components present in the system. Thus each of these systems would be difficult to adapt for metered dose delivery, in which a reproducible amount of liposome-encapsulated drug is needed. The present direct spray-dried drug/lipid powder, on the other hand, provides a convenient and efficient way to accurately deliver metered-drug doses.

Summary of the Invention

The present invention is directed to a process for direct spray-drying a drug/lipid powder composition. This process involves preparing an aqueous solution, having no phosphate buffer salts, containing a water-soluble drug, and preparing a lipid-containing ethanol solution. These two solutions are then mixed in a weight to weight (w/w) ratio in the range of about 1:1 ethanol:water to 5:1 ethanol:water, to form a solution mixture. The mixture is formulated to have between about 3%

to 4% weight to volume (w/v) total solids. This mixture is then spray dried and the resulting dried particle powder collected.

The above process can be used to generate drug/lipid powder compositions containing a variety of water-soluble drugs including: albuteral sulfate, ephedrine sulfate, ephedrine bitartrate, isoetharine hydrochloride, isoetharine mesylate, isoproteranol hydrochloride, isoproteranol sulfate, metaproteranol sulfate, terbutaline sulfate, procaterol, and bitolterol mesylate, atropine methyl nitrate, cromolyn sodium, propranolol, fluoroisolide, ibuprofen, gentamycin, tobermycin, pentamidine, penicillin, theophylline, bleomycin, etoposide, captopril, n-acetyl cysteine, verapamil, calcitonin, atriopeptin, α -1 antitrypsin, interferon, oxytocin, vasopressin, insulin, interleukin-2, superoxide dismutase, tissue plasminogen activator, plasma factor 8, epidermal growth factor, tumor necrosis factor, granulocyte/macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor, lung surfactant protein, and lipocortin.

The lipid containing ethanol solution must contain at least one membrane-forming lipid. Exemplary lipids for use in the powders of the present invention include the following lipids, their analogues and derivatives: phosphatidyl choline, phosphatidylglycerol, phosphatidic acid, phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine, sphingomyeline, and cholesterol. Representative analogues and derivatives of these lipids are presented in Table 1.

A preferred embodiment of the invention uses a lipid-containing ethanol solution containing phosphatidylcholine, phosphatidylglycerol, and α -tocopherol in a w/w ratio of about 167:15:0.1.

The process can also utilize other organic solvents, such as "Freons" which do solubilize lipids, and further include non-membrane forming lipids, such as cholesterol.

The drug/lipid powder composition of the present invention can be packaged into packets containing a selected dose of the drug.

The present invention also includes a number of methods for administering a water-soluble drug to the respiratory tract at a selected dose which involve producing an airborne suspension of the above described drug/lipid powder composition. There are three methods described for the delivery of the powder. In the first, the drug/lipid composition powder particles are suspended in a fluorocarbon propellant which does not solubilize the particles. The suspension is stored under pressure in a cannister and a selected amount of the suspension can be released from the cannister in an aerosolized form which is inhaled by the user. In the second and third method, the drug/lipid powder particles are provided in moisture-free packets, each containing a metered dose of the drug in the powder, and the particles are expelled from the packet in an air-borne form by either a propellant or an airstream produced by user inhalation. These methods of administering an drug by inhalation have been described in co-owned U. S. Application No. 4,895,719, herein incorporated by reference.

Also described in the present disclosure are a number of relatively portable and convenient apparatuses for administering the drug/lipid powder composition of the present invention, at a selected dose, via the respiratory tract.

Brief Description of the Figures

Figure 1 is a side cutaway view of a delivery system to be used with the direct spray-dried drug/lipid composition of the present invention. In this device the composition powder is contained in a capsule-like packet, and the packet is supported in the air passageway of a delivery device, for entrainment of the powder in a stream of air drawn through the passageway.

Figure 2 displays the data for encapsulation of albuterol sulfate versus volume of rehydration using the direct spray-dried powders.

Figure 3 shows the data for a linear regression of the log(percent encapsulation) versus volume of water used in rehydration of the direct spray-dried powders.

Figure 4 shows a comparison of spray-dried liposome encapsulated albuterol sulfate and Proventil™ effects on airways resistance response to histamine in guinea pigs after metered dose aerosol administration.

Detailed Description of the Invention

I. Preparation of the Direct Spray-Dried Drug/Lipid Composition

This section discusses methods of preparing the direct spray-dried drug/lipid composition of the present invention. This composition has a number of properties which make it valuable for use with inhalation therapies including: (a) the ability to form fine particle sizes on spray drying; (b) stability on storage in dehydrated or propellant suspension form; (c) high liposomal drug entrapment efficiency upon rehydration of the composition; and, (d) selected pharmacokinetic behavior when delivered to the respiratory tract.

The prior art spray-drying methods all require preformation of the liposome-encapsulated drug suspension, dilution, and then spray-drying of the liposome suspension (see, for example, U. S. Patent No. 4,895,719). The present invention has the important advantage that preformation of the liposome-drug suspension is not required.

Table 1

Lipid	Abbrev.	Charge	T _m (°C)
Egg phosphatidylcholine	EPC	0	-15 to -7
Dilaurylolphosphatidylcholine (C12:0)	DLPC	0	-1.8
Dimyristolphosphatidylcholine (C14:0)	DMPC	0	23
Dipalmitolphosphatidylcholine (C16:0)	DPFC	0	41
Distearylolphosphatidylcholine (C18:0)	DSFC	0	55
1-Myristoyl-2-palmitolphosphatidylcholine (C14:0, 16:0)	MPFC	0	27
1-Palmitoyl-2-myristoyl phosphatidylcholine (C16:0, 14:0)	PMPC	0	35
1-Palmitoyl-2-stearoyl phosphatidylcholine (C16:0, 18:0)	PFPC	0	44
1-Stearoyl-2-palmitoyl phosphatidylcholine (C18:0, 16:0)	SPFC	0	47
Dioleoylphosphatidylcholine (C18:1)	DOPC	0	-22
Dilaurylolphosphatidylglycerol	DLPG	-1	4
Dimyristolphosphatidylglycerol	DMPG	-1	23
Dipalmitolphosphatidylglycerol	DPPG	-1	41
Distearylolphosphatidylglycerol	DSPG	-1	55
Dioleoylphosphatidylglycerol	DOPG	-1	-18
Dimyristoyl phosphatidic acid	DMPA	-1	51
Dimyristoyl phosphatidic acid	DMPA	-2	45
Dipalmitoyl phosphatidic acid	DPFA	-1	67
Dipalmitoyl phosphatidic acid	DPFA	-2	58
Dimyristoyl phosphatidylethanolamine	DMPE	-	50
Dipalmitoyl phosphatidylethanolamine	DPPE	--	60
Dimyristoyl phosphatidylserine	DMPS	--	38
Dipalmitoyl phosphatidylserine	DPFS	--	51
Brain phosphatidylserine	PS	--	6-8°
Brain sphingomyelin	BSP	0	32
Dipalmitoyl sphingomyelin	DPSP	0	41
Distearyl sphingomyelin	DSSP	0	57

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The only requirement for lipid components to be used in the method of the present invention is that the lipids are able to form membranes in aqueous solutions. Lipids typically used in the present invention are egg phosphatidylcholine (EPC) and egg phosphatidylglycerol (EPG). Many other membrane-forming lipids can be used in the present invention either singly or in combination; a sampling of such lipids is presented in Table 1. In conjunction with the membrane-forming lipids, other lipid components, such as cholesterol can be used to formulate the drug/lipid composition. In general, cholesterol has a fluidizing effect in rehydrated liposomes of the present invention which containing predominantly saturated phospholipid components. The specific lipid composition of liposomes can affect the rate of drug release in the respiratory tract (reported in co-owned U. S. Patent No. 4,895,719).

The direct spray-dried drug/lipid composition of the present invention may also be formulated to include various types of drug-protective or lipid-protective agents, such as the antioxidant .alpha.-tocopherol, which is typically included at a mole ratio of between about 0.1-2.0 mole percent of total lipids.

A typical formulation for the present invention is partially hydrogenated EPC: Cholesterol: EPG: α -tocopherol in the mole ratio of 55:40:5:0.1 (Example 1). Cholesterol can be omitted from the formulations of the present invention (Example 3). Further, single lipids, such as EPC, have been effectively used as the sole lipid component of the drug/lipid composition of the present invention.

The lipid components of the composition are combined in a solvent which completely solubilizes them and which solvent is able to form a clear solution when mixed with an aqueous solution. A particularly useful solvent in this regard is ethanol. Ethanol is used as the primary solvent in all the Examples; in several of the examples

(Examples 1 and 2) a small quantity of "Freon 11" (CCl_3F) was used to facilitate the solubilization of the lipid components. Although a clear solution is desirable it is most important that (i) the lipid components be solubilized, (i) the liquid be free-flowing when it is used as a feed solution in the spray-drying process, and (iii) there is no solid precipitate in the feed line.

As defined herein, "drug" is intended to include any pharmacologically active agent which has a site of action in the respiratory tract or is therapeutically active when it is taken up systemically from the respiratory tract. Such drugs may include antibiotics, peptide hormones, enzymes, enzyme inhibitors, anti-tumor agents, bronchodilators, allergens, and antihistamines.

The most important class of drugs for use with the present invention are predominantly water-soluble drugs which tend to partition into the aqueous phase of a water/oil two-phase system. More particularly, this class of drugs tend to partition preferentially in the aqueous interior phase of liposomes, rather than in the lipophilic bilayer phase. Drugs in this class include relatively small, liposome-permeable drugs such as albuterol (salbutamol) sulfate, ephedrine sulfate, ephedrine bitartrate, isoetharine hydrochloride, isoetharine mesylate, isop4roteranol hydrochloride, isoproteranol sulfate, metaproteranol sulfate, terbutaline sulfate, procaterol, and bitolterol mesylate, atropine methyl nitrate, cromolyn sodium, propranolol, fluoroisolid, ibuprofen, gentamycin, bobermycin, pentamidine, penicillin, theophylline, bleomycin, etoposide, captopril, n-acetyl cysteine, and verapamil; and relatively large, liposome-impermeable drugs, such as peptide hormones, enzymes, enzyme inhibitors, apolipoproteins, and higher molecular weight carbohydrates, as exemplified by calcitonin, atriopentin, α -1, antitrypsin (protease inhibitor), interferon, oxytocin, vasopressin, insulin, interleukin-2, superoxide dismutase, tissue plasminogen activator (TPA), plasma factor

8, epidermal growth factor, tumor necrosis factor, granulocyte/macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor, lung surfactant protein, and lipocortin.

5 The drug of choice is dissolved in an aqueous solution at a specified concentration. Generally, the concentration of the drug is established relative to the amount of lipid solids present in the composition. In the Examples the ratio of albuterol sulfate to total
10 lipid was approximately 1:2.6 (w/w).

 The drug-containing aqueous solution is then mixed with the lipid-containing solution, resulting in the feed solution. It is important that the feed solution be free-flowing. For this reason it is important that the
15 aqueous solution be free of phosphate salts; the presence of phosphate buffer salts typically results in a cloudy precipitate after mixing the aqueous solution with the lipid-containing solvent. If the pH of the feed solution is a problem, then a different buffer salt might
20 be used in the formulation of the aqueous solution. If some haziness of the solution occurs, the temperature of the feed solution can be slightly elevated to prevent crystallization. Also, the ratio of lipid-containing solvent to aqueous solution can be adjusted to optimize
25 clarity. The presence of crystals in the feed solution creates problems with line clogging and effective atomization of the feed solution. Further, if the feed solution is not free-flowing the dried product may not be homogeneous if one or more components selectively precipitates before the solution reaches the atomizer.
30

 Feed solutions have been successfully spray dried at concentrations ranging from 3-4% total solids in suspension in the final feed solution. Drying at a concentration of 3.5% solids consistently yielded a good powder
35 product. Stickiness of the powder is also affected by the ambient humidity. To minimize the influence of ambient humidity either compressed air or nitrogen gas

were used as the source of heated inlet gas. The powder quality of samples can sometimes be improved by overnight vacuum drying. After additional drying, the powders become more free flowing and easier to work with for further measurements, such as percent encapsulation.

A number of commercially available spray-drying devices can be used to spray-dry the feed solution. Typically the spray-driers involve atomization of the feed solution into a large chamber and entrainment of the atomized solution in a moving stream of hot gas, such as heated compressed air or nitrogen gas. The feed solution is usually maintained at a constant temperature. The temperature of spray-drying must be sufficient to volatilize the liquid in the microscopic droplets. The remaining particles then either fall to the bottom of the chamber and are collected there, or are carried by the hot gas and collected by, for example, forcing the hot gas flow to form a cyclone causing even small particles to fall from the gas flow. The heated gas, which has been somewhat cooled at this point, is either released to the atmosphere or recycled through condensers. There are numerous configurations for atomizers and for directing the flow of hot gas. The two systems described in the present disclosure were the Buchi 190 mini-spray-drier, using an inlet temperature of 58-72°C and an outlet temperature of 34-42°C (Example 1), and the Niro 53 spray-drier, using an inlet temperature of about 165°C and an outlet temperature of about 70°C (Examples 1-3).

Example 1.2 discloses the characteristics of Buchi spray-dried powders using two water: ethanol: "Freon 11" solvent systems having the ratios of 25:70:5 and 14.3:80:5.7 (v/v/v). The percent encapsulation ranged from 69-80% and the moisture content of the powders were low, from about 1-3%. The data presented in Table 2 also show that the powders obtained by the method were generally flowable either directly after spray-drying or after a secondary drying performed under vacuum. The sticki-

ness of the powder has also been reduced by keeping the ambient humidity low or collecting the powder with immediate storage over Drierite™. Also the use of nitrogen as the source of inlet gas has repeatedly yielded flow-
5 able powders.

The data presented in Table 3 demonstrates that spray-drying using either the Buchi or Niro devices yielded very similar powder products with equivalent efficiencies of encapsulation. The results also illustrate that the process of the present invention can
10 readily be scaled-up for production of the drug/lipid composition.

The percent drug encapsulation achieved by the present invention is routinely about 70% or greater
15 (Tables 2 and 3). Typically the percent encapsulation is measured by adding water to the powder in a 2:1 weight ratio. Drug encapsulations for the direct spray-dried powders of the present invention were also determined in varying volumes of water, ranging from 100 ul (standard
20 condition) to 2000 ul per 50 mg of bulk powder. Results obtained are shown in Table 4 and semi-log plots of the data are shown in Figure 2. The data demonstrate an inverse proportion between volume of rehydration and apparent percent encapsulation.

25 Linear regression was performed on the straight-line segment of the curve in Figure 2, i.e., the range of 100-1000 ul volume of rehydration: the results are shown in Figure 3. The plot is linear in this range ($R=1.00$). Extrapolating the plot to zero rehydration volume yields
30 theoretical encapsulation of albuterol sulfate in the direct-dried powders of approximately 75%.

The present method generates a powder which, upon rehydration, yields liposome encapsulated drug at a comparable percent encapsulation to spray-dried preformed
35 liposomes. Example 2 (Table 5) presents data which compare the encapsulation efficiency of spray-dried preformed liposomes to the direct spray-dried drug/lipid

composition of the present invention. The data show that omission of the phosphate buffer salts from spray-dried preformed liposomes results in powders having essentially equivalent encapsulation efficiencies as the direct spray-dried drug/lipid powder (Table 5). The ability to produce the direct spray-dried powder in bulk and achieve encapsulation essentially equivalent to that achieved by the spray-dried preformed liposome powder, is an important advantage over the prior art methods; preformation of liposomes encapsulating the drug before spray-drying is eliminated.

The direct spray-dried drug/lipid composition of the present invention can be formulated, as mentioned above, using a variety of lipids. The only requirement for the primary lipid of the composition is that it be a membrane-forming lipid. Example 3 describes a phosphatidylcholine/phosphatidylglycerol lipid formulation of the drug/lipid composition. This formulation has proven effective in repeated large scale runs to generate the direct spray-dried powder. Only ethanol was used as the lipid-dissolving solvent in Example 3; there was no addition of "Freon".

As can be seen from the data presented in Table 7 this drug/lipid composition dries well and has approximately the same residual moisture as the formulations containing cholesterol. The percent encapsulation of albuterol sulfate was approximately 70%. The mass median diameter of the dried particles, as determined by static laser ensemble light scattering, had a bimodal distribution with peaks at about 1 and at 7-10 microns with a distribution over the range of about 0.1 to 20 microns. The optimal size for respirable particles is less than about 5 microns. The majority of the particles of the direct spray-dried drug/lipid powder fall well within this range. Upon rehydration of the powder, the bimodal distribution of the dried particles is replaced by a normal distribution of liposomes having a single-peak at

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approximately 1.5 microns. Accordingly, the composition of the present invention is well suited for use in inhalation therapies and respiratory tract administration of systemic drugs.

5

II. Delivery Devices

This section describes three self-contained delivery devices designed for producing an airborne suspension of the direct-dried drug/lipid powder of the present invention. As defined herein "self-contained" means that the powder aerosol is produced in a self-contained device that is propelled by a pressure differential created either by release of a pressurized fluorochlorocarbon propellant or by a stream of air drawn through or created in the device by the user. An important feature of delivery devices using pressurized fluorochlorocarbon propellant is that the powder is insoluble in the propellant, ie. the powder particles remain intact.

The devices described in parts A and B both employ propellants for aerosolization; the device of part C employs a stream of air drawn through the device by the user. All of these devices have been described in co-owned U. S. Patent No. 4,895,719, herein incorporated by reference.

25

A. Drug/Lipid Powder Particle Suspension in Propellant

This system uses a conventional pressurized propellant spray device for delivering a metered amount of the direct-dried drug/lipid composition powder which is suspended in the propellant. Since the system requires long-term suspension of the powder in a suitable propellant, the powder and propellant components of the suspension must be selected for stability on storage. To this end, experiments performed in support of the present

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invention indicate that the lipids used to generate the powder are preferably partially or totally hydrogenated lipids.

As mentioned above, an important consideration in the selection of propellants is that the drug/lipid powder particles remain intact. Several fluorochlorocarbon propellants which meet this requirement are particularly suitable for use with the powders of the present invention including "Freon 113" ($\text{CCl}_2\text{FCCLF}_2$) and "Freon 115" (CClF_2CF_3). It has been disclosed in co-owned U. S. Patent No. 4,895,719 that these propellants cause the least disruption of spray-dried pre-formed liposomes.

To form the powder propellant/suspension, the direct spray-dried drug/lipid powder is added to the selected propellant to a final concentration of about 10-25 weight percent of powder to total propellant. Further, the final concentration of drug/lipid powder in the propellant is adjusted to yield a selected metered dose of the drug in a given aerosol suspension volume. Thus, for example, if the powder is formulated to contain 0.05 mg drug per mg direct-dried powder, and the selected dose of drug to be administered is 1 mg, the suspension is formulated to contain 20 mg of powder per aerosol dose.

The suspension is packaged in a conventional self-contained propellant device, such as described in co-owned U.S. Patent No. 4,895,719. The device generally includes a replaceable propellant cartridge which holds the propellant suspension under a suitable aerosolizing pressure. The cartridge is equipped with a metering valve which is designed to release a selected volume of the suspension, under pressure, when the valve is activated by being pushed inwardly. The cartridge is supported in a conventional atomizer which functions to aerosolize the suspension released from the cartridge, on valve actuation, and direct the aerosol in a stream toward the user's mouth, which may engage the mouthpiece of the atomizer. Aerosol delivery devices of this type are well

known.

The aerosol particles which are initially formed are propellant droplets containing the powder in suspended form. As these droplets are propelled through the air, the propellant rapidly evaporates, leaving an airborne suspension of the powder particles containing a selected dose of drug. When the powder particles come in contact with moisture, e.g. on mucosal tissue, liposomes encapsulating the drug are spontaneously formed.

10 B. Drug/Lipid Powder Entrainment in a Propellant

In this system, direct-dried drug/lipid powder containing a metered-dose quantity of drug is prepackaged in dehydrated form in a delivery packet. The packet is used with a propellant spray device, to eject the powder contents of the packet in an airborne suspension of liposome particles; such a system is described in co-owned U.S. Patent No. 4,895,719. The packet contains a quantity of direct-dried drug/lipid powder containing a metered dose of a selected drug. The packet, when supplied to the user, is sealed at its opposite ends to keep moisture out, and the powder is loosely held in the tubing. The seal is preferably a conventionally formed thin polymer barrier which is easily rupturable at each tubing end to create an unobstructed passageway through the tubing.

The system also includes a propellant spray device which contains a charge of pressurized propellant, such as a "Freon," and which is equipped with a valve for releasing a stream of aerosolized propellant. As in Section A above the "Freon" is chosen so the powder particles remain intact while they are in contact with the "Freon." The downstream end of the valve terminates in a nozzle which is adapted to receive an end of the packet to attach the packet operably to the device.

35 In operation, a packet is attached to the nozzle, preferably by inserting a sealed end of the packet tubing over the nozzle, to break the seal. The seal at the free

end of the tubing is also punctured, for example, in the case of a friable seal, by pinching the tube end of the tubing. To deliver the powder charge in the packet, the valve in the spray device is actuated briefly. It can be appreciated that the powder in the packet becomes entrained in the stream of aerosolized propellant passing through the tubing. The propellant in turn rapidly evaporates, yielding an airborne suspension of the powder. Administration of the powder occurs, as above, by the user drawing in breath at the same time the powder suspension is delivered. It will be understood that in its commercial embodiment, the device is supplied as a spray device with a plurality of powder packets, one for each dosing.

15 C. Liposome Entrainment in Air

A third type of delivery system uses an airstream produced by user inhalation to entrain the drug/lipid powder particles and draw them into the user's respiratory tract. One exemplary system, based on a known type of aerosolizing device, is shown at 42 in Figure 1. The drug/lipid powder in this system is provided in moisture-free packets, such as packet 44, each containing a metered dose of the drug in the powder.

A delivery device 46 in the system includes a curved pipe 48 which defines an inlet passageway 50 terminating at a reduced diameter nozzle 52 which is dimensioned to receive and hold packet 44, as shown. The other end of the pipe confines a convection region 54 where the powder drawn out of the packet is distributed throughout the cross-section of the downstream end of the pipe, while being drawn into the user's respiratory tract by inhalation. Air convection in the downstream pipe region is created by a paddle 56 which is freely rotatable on a shaft 58 supported within region 54. The pipe is provided with a mouthpiece 60 at its downstream end.

In operation, a packet is placed on the nozzle, preferably in a manner which ruptures the seal at the

"inner" end of the packet, as above, and the other end of the packet is unsealed. The user now places his lips about the mouthpiece and inhales forcefully, to draw air rapidly into and through the pipe. As can be appreciated in Figure 1, the air drawn through the pipe becomes concentrated at the nozzle, creating a high-velocity airstream which carries the powder out of the packet and into the convection region. The airstream and entrained powder impinge on the paddle, causing it to rotate and set up a convection within region 54. The powder is thus distributed more evenly, and over a broader cross-section, just prior to being drawn into the user's respiratory tract by the inhalation. The powder spontaneously rehydrates to form liposomes encapsulating the drug.

As with the above-described systems, the system here is supplied to the user in the form of a single aerosol pipe with a plurality of single-dose packets.

III. Therapeutic Applications

A. Treatment of Bronchoconstriction

The present invention is useful in treating a variety of bronchoconstriction conditions, such as bronchial asthma, emphysema, bronchitis, and bronchiectasis. These conditions are responsive to β_2 -agonists, which are also useful in treating peripheral vascular disease and shock, and may be used to delay delivery in cases of premature labor.

Earlier studies, reported in the co-owned, co-pending patent application for "Liposome Bronchodilator Method and System" (Serial No. 366,299, filed 6/13/89), have demonstrated three important advantages of liposome administration of β_2 -agonist drugs to the respiratory tract. First, undesired side effects of the drugs, due to rapid systemic drug uptake, are largely eliminated or reduced, due to the much lower drug levels in the bloodstream which are observed after drug administration. In particular, "drug spiking" effects due to a large initial

surge of systemic drug, and which are responsible for noticeable tachycardia, dizziness, and other β_2 -agonist side effects, are largely avoided.

Another important advantage is that the drug, when
5 administered in therapeutic dose via liposomes, shows more effective bronchodilation, at times greater than about 1 hour after drug administration, than a comparable amount of drug given in free form. This means that effective relief from asthma or other bronchoconstriction
10 condition is provided with each administration, fewer administrations are needed, and the patient is subject to less drastic swings in the β_2 -agonist levels in the body.

Thirdly, the rate of drug release from the site of administration in the lungs can be modulated, according
15 to the lipid composition of the liposomes.

Delivery of drugs in a predominantly liposome-associated form solves a variety of problems encountered when the drug is administered in free or predominantly free form. In addition to the drug moderating and sustained-release features, the liposomes act to protect the drug
20 from oxidation and protect the respiratory tract from potentially irritating drugs, particularly those which, because of solubility properties, must be administered in micronized form.

The above-cited advantages for the use of liposome encapsulate drug also apply to the spray-dried compositions. Example 4 describes the procedures used to compare the bronchodilator and cardiovascular effects of a suspension of spray-dried liposomal albuterol sulfate
30 versus a commercially available microcrystalline suspension of free base albuterol (ProventilTM). Figure 4 graphically illustrates the data (Table 8) showing that the spray-dried liposomal albuterol sulfate provides longer term effects of albuterol sulfate, up to at least
35 2 hours, as compared to microcrystalline administration of free base albuterol sulfate (less than 1 hour). Further, the data in Table 9 show that there was no sig-

nificant difference in the cardio/pulmonary effects of albuterol independent of method of administration.

These results illustrate that administration of a spray-dried composition is effective to achieve the above-cite advantages of liposome-encapsulated drug administration.

A variety of drugs effective in the treatment of bronchial or pulmonary conditions can be administered in the present system, with the same advantages. For example, for intrapulmonary drug delivery, α -1-protease inhibitor is delivered to the pulmonary interstitium in liposome-encapsulated form to stem the development of pulmonary emphysema. The liposomes act to protect the protease inhibitor's tertiary structure from oxidation, and facilitate its transport across the pulmonary cell membranes.

The present invention provides a convenient, self-contained system for administering a direct spray-dried drug/lipid composition to the respiratory tract in a metered dose form. Some of the drug (eg., Table 7, the approximately 30% non-encapsulated drug) will be available for rapid utilization in free drug form, versus that which is released slowly from the rehydrated liposomes over a several-hour period.

Another advantage of the system is that a relatively constant and predictable drug dose can be administered with each dosing, since the amount of lipid and entrapped drug remains substantially constant from one administration to another. The system is also convenient to use, since only a small, self-contained device -- no larger than the aerosoling devices described in Section II -- is needed.

B. Systemic-Action Drug Delivery

Many drugs whose primary mode of action requires uptake into the bloodstream can also be delivered by inhalation. The inhalation route allows the drug to be delivered rapidly to the bloodstream, is much more conve-

nient to the patient than intramuscular or intravenous injection, and is suitable for drugs which cannot be delivered orally because of drug stability of the like. Heretofore, drug delivery by inhalation has been limited
5 by a number of factors, including (a) lack of control of total drug dose, (b) inability to achieve long-term or controlled-released drug delivery, (c) the requirement for expensive and cumbersome inhalation equipment for drugs which cannot be suspended in free form in a propellant solvent or are otherwise unstable in free form, (d)
10 the extremely unpleasant taste of some drugs, and (e) drug irritation to the respiratory tract.

The present invention overcomes or minimizes many of the problems which have limited systemic drug delivery by inhalation heretofore. The problem of regulating drug
15 dose is the most difficult problem to resolve. Using the present drug/lipid composition there will be some variability, from dose to dose, in the amount of aerosolized material which actually reaches the respiratory tract at a level which allows systemic uptake. However, some
20 aspects of the direct dried drug/lipid composition are designed to facilitate more consistent total uptake. The lipids themselves function as a bulking agent, i.e., solid particle carrier or filler, and given that the drug
25 can be entrapped in the powder particles at high concentration, much less total particulate material may need to be administered. Accordingly, less drug would be lost in "non-target" areas of the mouth and throat. Secondly, delivering a drug in particulate form from a propellant aerosol favors good drug uptake, and the present system
30 allows many drugs to be administered in this form.

As indicated above, the direct-dried drug/lipid composition provides controlled-release systemic uptake, and the rate of uptake of a water-soluble drug can be
35 controlled to some extent by the lipid composition of the liposomes.

The system of the invention allows water-soluble drugs to be delivered from a convenient, relatively inexpensive self-contained delivery device. As indicated above, the drug in lipid-entrapped powder form may be more stable and less irritating to the respiratory tract when administered. Further, the taste of the drug may be substantially masked in lipid-entrapped powder form.

An example of a specific application for systemic drug delivery is the encapsulation and delivery of nitroglycerin, a coronary vasodilator used to relieve the symptoms of angina pectoris. The drug formulation typically will contain about 30% free drug, which can be rapidly absorbed by the pulmonary bloodflow and transported directly to the heart, its primary site of action, to provide immediate relief from the chest pain associated with angina. The remaining 70% or more of the liposome-encapsulated drug which is liposome encapsulated upon rehydration is released slowly, at a rate controlled by liposome composition, to afford prolonged coronary vasodilation, and thus relief from chest pain for an extended period.

Oxytocin, a peptide hormone that induces and augments the strength of uterine muscle contractions during labor, can be formulated and delivered in a manner similar to that described for nitroglycerin. It is currently delivered by intravenous infusion, a process that requires placement and maintenance of a venous cannula, a sometimes difficult procedure that limits the patient movement and posture. Aerosols of direct spray-dried lipid-oxytocin powders would provide immediate and sustained delivery to the systemic circulation, similar to that provided by IV infusion, without restricting patient motion. A wide range of other systemic-acting drugs, such as those named above in Section I, could also be administered advantageously by the system of the invention.

While preferred embodiments, uses, and methods of practicing the present invention have been described in detail, it will be appreciated that various other uses, formulations, and methods of practice as indicated herein are within the contemplation of the present invention.

EXAMPLE 1

Formulations and Characteristics of the Direct Spray-Dried Drug/Lipid Composition

10 This example describes the basic formulation of and general procedures for the production of the drug/lipid composition.

1.1 Formulation of the Direct Spray-Dried Drug/Lipid Composition

15 The spray drying runs described in this example were conducted using the following lipid composition: PHEPC IV-40: Cholesterol: EPG: α -tocopherol / 55:40:5:0.1 (mole ratio). The ratio of albuterol sulfate to total lipid was approximately 1:2.6 (w/w). The solvent composition
20 for direct drying in this example was the single-phase solvent system water/ethanol/Freon 11 (v/v/v ratios are given in the footnote to Table 2). The solutions were spray-dried at a final concentration of 3.5% total solids in solution.

25 The drug was dissolved in water, the cholesterol in Freon and the remaining remaining lipids in ethanol. The lipid solutions were combined, followed by addition of the aqueous drug solution. This final solution was clear and used as the feed solution for spray-drying. The feed
30 solution was stable and did not form precipitates for at least 24 hours at room temperature.

Compressed air was used for atomization of the feed solution and for the heated inlet air in order to standardize conditions. The air used for spray drying should
35 be at a consistent humidity to reproducibly generate free flowing powders on days with different ambient weather conditions.

Small scale spray drying of the drug/lipid composition was accomplished in the Buchi 190 mini spray drier (Buchi Laboratoriums Technik AG, CH-9230 Flawil, Switzerland), using a gas flow setting of 700 Nl/h (nominal
5 liters/hr), 1-2 ml/min sample input of the feed solution and inlet/outlet temperatures of 58-72°C/34-42°C.

Larger-scale spray drying runs were accomplished using a Niro model 53 portable spray drier (Niro Atomizer, Copenhagen, Denmark); drying was performed with an
10 inlet temperature of about 165°C, an outlet temperature of about 70°C, a gas flow setting of 800-1100 feet per minute and an input rate of the feed solution of 28-32 ml/min.

The powders generated by spray-drying were formulated to yield total lipid 680 mg/g and albuterol sulfate 275 mg/g. In general, the nominal composition of the
15 powders were as follows (values given as milligram compound per gram total powder): albuterol sulfate 274 mg/g; phosphatidylcholine 500 mg/g; phosphatidylglycerol 45.4 mg/g; cholesterol 181 mg/g; and, .alpha.-
20 tocopherol 0.5 mg/g.

1.2 Characteristics of the Buchi Spray-Dried Compositions

25 A number of characteristics of the spray-dried powders have been examined (see Table 2). The percent encapsulation of the spray-dried bulk powders was routinely measured as follows. The powder was rehydrated using twice its weight in water (typically 50 mg of bulk
30 powder in 100 ul of deionized water) and allowed to sit for several hours. The suspension was mixed intermittently on a vortex mixer for approximately two hours before being left to rehydrate overnight. This suspension was then diluted in isotonic buffer and the liposomes pelleted by centrifugation. The supernatant was
35 removed. Both the supernatant and the pellet were assayed for drug content: for albuterol sulfate UV absor-

bance at 275nm was measured and the concentration of the drug was calculated using $\epsilon=6.314$ as the extinction coefficient. The percent encapsulation was then calculated by dividing the concentration of the drug in the pellet by
5 the total amount of drug present (supernatant plus pellet) and multiplying by 100.

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Table 2
 Characteristics of Spray-Dried Albuteral Sulfate/Lipid
 Powders Generated by Direct Drying on Buchi 190

Sample (Notebook Ref)	Solvent Mixture ^a	Encapsu- lation (%)	% H ₂ O	Visual Appearance ^c e	d
375-PYY-44	A	69	ND	Flowable	ND
424-KF-6	B	76 ± 2	ND	Sticky	Drier
424-KF-11	B	80 ± 2	ND	Sticky	Drier
424-KF-32	B	74 ± 1	1.16 ± 0.06 ^e	Sticky	Drier
424-KF-38	B	NA	1.71 ± 0.05 ^e 0.99 ± 0.101 ^b	Sticky	Flowable
424-KF-50	B	80 ± 5	2.77 ± 0.03 ^e 0.59 ± 0.06 ^e	Sticky	Flowable
Mean ^a		77			
Std.Dev.		± 3			

a Solvent mixtures used were H₂O:EtOH:Freon 11; A = 25:70:5
 (/v/v), b = 14.3/80/5.7 (v/v/v).
 b All powders were white.
 c Without secondary drying
 d After secondary drying
 e Mean for samples made using solvent mixture B.
 NA Data not available.
 ND Not determined.

The water content of the powders was determined by Fisher.

Powders were also assessed for visual appearance, including description of color and whether or not they were flowable.

As can be seen from the data presented in Table 2 the drug/lipid composition gave drug encapsulation efficiencies in the range of 69% to 80% and moisture contents of less than 2.7% or less. Further, the powders were white and generally flowable. If the direct-dried powders were sticky they were further dried by overnight vacuum drying. After this additional drying the powders became more free flowing and easier to work with for other measurements, such as percent encapsulation.

1.3 Comparison of the Spray-Dried Products Generated by the Buchi and Niro Spray Driers

Comparisons of direct-dried drug/lipid compositions using both the Buchi 190 and Niro 53 spray driers are shown in Table 3. The formulation of the feed solutions was as above; the solvent composition was water: ethanol: "Freon 11" at the ratio of 14.3: 80: 5.7 (v/v/v).

Table 3

Powder Composition and Characteristics of Representative Direct-Dried Powders Generated on the Buchi 190 and Niro 53 Instruments

Component or Characteristic	NOMINAL OBSERVED		
	Direct Dried		
	Buchi	Niro	
Appearance	white, flowing	white flowing	cream color flowing
ABS	275	276	273
% Encapsulation	>50	69	70
P-lipid Phos. (mg/g)	20.4	27.1	22.8
PC (%)	91.7	89.4	ND*
PG (%)	8.3	6.1	ND*
LPC (%)	0	1.6	ND
pH	Depends on buffer	3.4	3.6
Water Content (%)	1.9	5.3	1.0

* ND - Not Determined

These comparisons show that powders obtained from the Buchi and Niro were very similar for most characteristics measured. One observed differences was the higher water content in the Niro direct-dried powder. This difference did not appear to affect other characteristics.

The spray drying run conducted using the Niro spray dryer produced a fine powder which was collected into a jar and placed over Drierite™ immediately after

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collection. Scanning electromicroscopic analysis indicated that the powder particles were 2-3 um in diameter. Encapsulation of albuterol sulfate after rehydration was 70%.

5 These results confirm the high encapsulation of drug
in powders generated by direct drying. The results also
indicate that the process can be scaled up.

1.4 Varying the Rehydration Volume

Drug encapsulations were determined following
10 rehydration in varying volumes of water, ranging from 100
ul (standard condition) to 2000 ul per 50 mg of bulk
powder. Results obtained are shown in Table 4 and semi-
log plots of the data are shown in Figure 2.

Table 4

Encapsulation of ABS Versus Volume of Rehydration Direct-Dried Powders

	Volume of Water Added (ul)	Percent Encapsulation Direct-Dried Powder
20	100	69
	200	57
	400	43
	1000	21
25	2000	17

The data demonstrate a dramatic inverse proportion
30 between volume of rehydration and apparent percent
encapsulation. The powder shows a significant drop in
encapsulation as the volume of rehydration is increased.

Linear regression was performed on the straight-line segment of the curve in Figure 2, i.e., the range of 100-35 1000 ul volume of rehydration. The results are shown in Figure 3; the plot is linear in this range ($R=1.00$). Extrapolating the plot to zero rehydration volume yields

theoretical encapsulation of albuterol sulfate in the powders of 75%.

EXAMPLE 2

5 Comparison of Percent Drug Encapsulation by Spray-dried
 Pre-formed Liposomes and Spray-Dried Drug/Lipid
 Compositions

 This example compares the drug/lipid compositions of the present invention to prior art methods for generating
10 spray-dried liposome compositions. The lipid composition, drug content, and solvent formulations were essentially as described in Example 1.

2.1 Preparation of Spray-Dried Liposome Compositions

15 The lipid components were weighed out, co-dissolved in chloroform and dried down on the walls of round bottom flasks (e.g., U. S. Patents Nos. 4,766,046 and 4,483,929). A 10 mM Phosphate Buffer Composition hydration buffer, including the albuterol sulfate, was
20 used in place of water as the aqueous solvent. The hydration buffer was then added and the thin films were hydrated with constant mechanical agitation at 45°C for one hour. The resulting liposome dispersions were sized by high pressure extrusion through 0.2 μ Nuclepore
25 membranes (Nuclepore, Calif.). Unencapsulated drug was removed from all liposome preparations by ultracentrifugation with an iso-osmotic phosphate buffer. The resulting liposome pastes (LP-phosphate) were then stored at 2-8°C in butyl rubber stoppered glass serum
30 bottles under nitrogen.

 A second similar composition was produced using water, including albuterol sulfate, as the hydration buffer (LP-water).

35 2.2 Spray-Drying the Pre-formed Liposomes

 The liposome pastes LP-phosphate and LP-water from 2.1 were diluted in, respectively, isotonic phosphate

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buffer or water. Individually, the resulting liposome suspensions were combined with the ethanol/freon solvent system to yield a final concentration of 3.5% solids. These feed solutions were then dried using the Niro spray-drier as described in Example 1.1.

2.2 Comparison of Percent Encapsulation of Spray-dried Liposomes versus the Spray-dried Drug/Lipid Composition

The drug/lipid composition was produced as described in Example 1. The spray-dried liposomes (LP-phosphate and LP-water) and drug/lipid (D/L-comp) compositions were all rehydrated as described in Example 1.2 and the percent encapsulation achieved with each composition is given in Table 5.

Table 5

	Sample	Percent Encapsulation*
20	LP-phosphate	52/53
	LP-water	70/71
	D/L-comp	70/74

(* duplicate measures)

The results show that the drug/lipid composition of the present invention gave encapsulation efficiencies equivalent to pre-formed liposomes when the liposome pastes were made without phosphate buffer salts.

EXAMPLE 3

Phosphatidylcholine/phosphatidylglycerol/albuterol sulfate

Spray-Dried Drug/Lipid Compositions

This example describes a lipid/drug formulation that does not contain cholesterol and is effective in forming liposome-encapsulated drug compositions upon rehydration. The spray-drying procedure was as described in Example 1 for use of the Niro Spray-drier with the exception that nitrogen was used as the inlet air source in place of

compressed air.

3.1 Composition of the Drug/Lipid Feed Solution

The drug/lipid feed solution contained the components listed in Table 6.

5

10

Table 6

Component	Supplier	mg/g Feed Solution	Total grams required
Albuterol Sulfate	Agvar Chemical	12.3	98.1
PHEPC TV40	Asahi Chemical	20.09	167.1
96% Egg PG	Asahi Chemical	1.82	14.6
Alpha tocopherol	Hoffman-La Roche	0.0223	0.178
Ethanol, Dehydrated, USP	U.S. Industrial	794.0	6352.0
Water, Purified, USP	Kendall McGaw	171.0	1368.0
Total:		1000.0	8000.0

20

As shown in Table 6 a typical quantity for a spray-drying run was 8 kg. The feed solution had a total of 3.5% solids (w/w) and a weight ratio of ethanol to water of 4.64:1. This formulation contained no Freon.

The nominal analyte concentrations in the feed solution were as follows: albuterol sulfate, 12.3 mg/g feed and total phosphorous 904.5 µg/g feed.

30

3.2 Characterization of the Spray-Dried Drug/Lipid Compositions

Table 7 summarizes the data collected to characterize the spray-dried drug/lipid compositions.

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Table 7
Powder Specifications

	Assay	Powder Specifications	
		Invention	IND
5	Identity	Retention Time Same as ABS)	
	Albuterol Sulfate (HPLC)	343 ± 17 mg/g	343 ± 34 mg/g
	Total Phosphorus	25.3 ± 2.53 mg/g	25.3 ± 2.53 mg/g
	Phospholipid Identity		
	Phosphatidylcholine	Complies	Complies
	Phosphatidylglycerol	Complies	Complies
	α-tocopherol identity	Complies	Complies
	Residual Moisture (Fisher)	NMT 2%, (20mg/g)	NMT 2.5%
10	pH, 3% in distilled water	3-5	TBD**
	Residual Ethanol (GC/MS)	NMT 500 ppm	NMT 500 ppm
	Percent Encapsulation	70 ± 7%	70 ± 10%
	Microbial Units	NMT 100 CFU/g	NMT 100 CFU/g
	Mass Median Diameter	1.5μ	TBD

*Not More Than

**To Be Determined

The concentration of albuterol sulfate was determined using HPLC analysis under conditions which solubilized the liposomes and freed the albuterol sulfate. The peak corresponding to the freed albuterol sulfate was quantitated by comparison to known concentrations of commercially available albuterol sulfate. The identity of the albuterol sulfate was determined by comigration of the freed albuterol sulfate with commercially available albuterol sulfate.

Total phosphate was determined by the Bartlett assay (Bartlett, 1959). The identities of the phospholipids and .alpha.-tocopherol were determined by thin-layer chromatography and comparison to commercially available standards. The residual moisture content of the powders was determined by the method of Fisher. The residual ethanol concentration was determined by gas chromatography. The microbial limits were determined by plating serial dilutions of the rehydrated drug/lipid compositions onto solid agar rich media (eg., LB, Maniatis et al.) and counting the colony forming units (CFU).

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The mass median diameter of the powder particles of the direct-dried drug/lipid powder and of liposomes resulting from the rehydration of the powder were determined using static laser ensemble light scattering (Malveen Mastersizer II, Malveen Instruments, Southborough, MA). The mass median diameter of the dried particles was 5 microns, however, the distribution of particle sizes was bimodal with peaks at approximately 1 micron and 7-10 microns. Upon rehydration a normal distribution having single peak appears at approximately 1.5 microns.

EXAMPLE 4

Bronchodilator and Cardiovascular Effects of a Suspension of Spray-Dried Liposomal Albuterol Sulfate

This example compares the bronchodilator and cardiovascular effects of a microcrystalline suspension of albuterol free base (Proventil™ inhaler, Schering) and a suspension of spray-dried liposomal albuterol sulfate (MDL-ABS).

4.1 Preparation of the metered-dose albuterol sulfate

A metered-dose inhaler form of albuterol was provided by a commercial preparation (Proventil™ Inhaler, Schering Corp., Kenilworth, NJ, Lot # 8 BBS 54). This product contained albuterol in its base form, and provided nominal delivery of 90 µg albuterol free base (equivalent to 108 µg albuterol sulfate).

A metered-dose inhaler form of liposomal albuterol sulfate (MDI-L-ABS) was prepared in several steps. Liposome-entrapped albuterol sulfate (LP-water) was produced as in Example 2.1 with the exception that .alpha.-tocopherol was omitted. The concentrate was then diluted in aqueous buffer and spray dried to obtain a free-flowing liposomal powder. The albuterol sulfate was 273 mg/g, and the phospholipid concentration was 709 µmol/g (total lipid concentration being 1180 µmol/g). This powder was formulated with propellant and filled

into metered-dose vials (3M/Riker). Delivery of albuterol sulfate from the metered-dose inhaler valve was determined spectrophotometrically to be 111 µg per actuation.

- 5 Histamine diphosphate (Sigma Chemical Co., St. Louis, MO)
was prepared daily in 0.9 saline as a 50 µg/mL solution and stored on ice until used.

10 4.2 Test Animals and Measurement Methods

Male Hartley strain guinea pigs (*Cavia porcellus*), 4-7 weeks old, weighing 350-500 g, were supplied by Harlan Sprague Dawley, Inc. After delivery from the vendor they were weighed and quarantined in pairs for at
15 least 3 days and reweighed. Water and Purina Guinea Pig Chow were given and the animals were allowed to feed ad libitum. Animals showing weight loss or overt signs of disease were not used in the studies. They were assigned to test groups on a random basis.

- 20 Pulmonary and Cardiac Function were measured as follows. The guinea pigs were anesthetized with an intramuscular injection of xylazine:ketamine (2.5:75 mg/kg). The trachea, esophagus, left common carotid artery, and left jugular vein were exposed and cannulated
25 with polyethylene tubing. The guinea pigs were then placed in a supine position in a whole body plethysmograph. The tracheal cannula was connected to the exterior to allow breathing from the room air. Changes in thoracic volume produced airflow into or out
30 of the plethysmograph. This flow was measured with a Validyne MP-45 differential transducer, which detected the pressure drop across the plethysmograph's constant resistance element. The flow signal was automatically integrated to produce a measurement of tidal volume.
35 Thoracic pressure was measured indirectly with the esophageal cannula and a Statham P23Dc fluid-filled transducer. Systemic arterial blood pressure (diastolic)

was measured from the catheter in the common carotid artery via a Statham P23Db transducer. All transducers were attached via Validyne carrier demodulators to a buxco Model 6 Pulmonary Mechanics Analyzer. The analyzer used the airflow and esophageal pressure to compute pulmonary resistance (R_L) at points of equal tidal volume. Data were converted from analogue to digital form and recorded on a Texas Instrument Data terminal.

4.3 Administration of the Metered Dose Albuterol Sulfate

For administration of metered-dose inhaler (MDI) aerosols, a cylindrical dosing chamber (3.8 cm diameter by 13.5 cm length, approximately 150 cc volume) was inserted between a Harvard rodent respirator and the animal in the plethysmograph. at the tracheal cannula. MDI vials were actuated four times into the dosing chamber. The dosing was followed by adjustment of the respirator to produce a stroke volume of 2 mL/100 g body weight and 60 breaths per minute. Actuation of the solenoid valves closed the normal breathing path (to room air) and opened the alternate path to the ventilator/nebulizer. Exposure to the aerosol in this fashion was carried out for two minutes. Atomized saline was used as a control.

The test animal was placed in the whole body plethysmograph, and basal data on pulmonary mechanics and cardiovascular state were collected. Then, the responses to an intravenous injection of histamine (10 μ g/kg) were determined as follows. Fifteen minutes after the initial histamine challenge, the animal was exposed to the aerosolized test substance (ProventilTM or MDI-L-albuterol sulfate) for two minutes, with the mechanical ventilation described above. Following this administration (designated the zero time point), the animal was given additional histamine challenges as above at 15 minutes, 1, 2, 3, and 4 hours.

4.4 Analysis of Results

All lung resistance responses (R_L) to the histamine were calculated as the maximum change from prehistamine levels. A one-way analysis of variance (ANOVA, Statsoft, Tulsa, Oklahoma) was used to test the hypothesis that there were no significant differences in R_L means between groups before the administration of a test substance (at - 15 minutes).

ANOVA was also used to test for significant differences in means for R_L between each time point within each group, and between each group at each time point. When ANOVA indicated a difference, a Student's t test was performed to locate the data sets showing the differences.

The cardiovascular responses (diastolic blood pressure) to the inhaled test substances were assessed by comparing the cardiovascular data directly before drug administration (after the first baseline histamine challenge) with the data collected during the following 10-minute period (before the second histamine challenge). The significance of any alteration was tested using ANOVA and post hoc Least Significant Difference (LSD) tests.

All graphed and tabulated data show the mean values plus or minus one standard error of the mean. A probability of $p < 0.05$ was accepted as significant.

For the ProventilTM and MDI-L-ABS aerosols, a large inhibition was measured for both dosage forms at the 15 minute histamine challenge, but by one hour the response

for Proventil had returned to the pre-drug administration level. For MDI-L-ABS, the inhibition response was statistically significant for up to two hours (see Table 8, and Figure 4).

5

Table 8
Inhibition of Bronchoconstriction with Metered Dose
Inhalation of Proventil and Liposomal Albuterol Sulfate¹

10

		<u>Resistance (cm/H₂O/mL/sec)</u>					
		Time After Metered Dose Aerosol Administration					
		-15 min	+15 min	1 hour	2 hour	3 hour	4 hour
Proventil n=5		0.27 ±0.11	0.09 ±0.05	0.23 ±0.11	0.32 ±0.14	0.36 ±0.13	0.26 ±0.15
% of baseline	100		32 ±6	86 ±8	140 ±36	153 ±42	110 ±34
MDI-ABS n=5		0.49 ±0.07	0.05 ±0.03	0.17 ±0.12	0.36 ±0.19	0.47 ±0.21	0.51 ±0.15
% of baseline	100		10 ±2	35 ±11	73 ±19	97 ±21	105 ±18

20

¹The increase in lung resistance (R_L) response to a bronchoconstrictor challenge of intravenous histamine (10 µg/kg) 15 min before and 15 min and 1, 2, 3 and 4 hours after inhalation of saline, Proventil (89 µg/actuation albuterol free base, or the equivalent 108 µg/actuation albuterol sulfate), and metered dose liposomal albuterol sulfate (92 µg/actuation albuterol free base, or the equivalent 111 µg/actuation albuterol sulfate). Values are means ± one SE.

25

Also, there was no statistically significant difference between the blood pressures measured after Proventil or MDI-L-ABS administration (Table 9).

35

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Table 9
Effect of Metered Dose Aerosols of Proventil and
Liposomal Albuterol Sulfate on Diastolic Blood Pressure¹

	<u>Minutes After Inha- lation of Aerosol</u>	<u>Diastolic Blood Pressure (mmHg)</u>	
		<u>MDL-ABS n=5</u>	<u>Proventil n=5</u>
10	-1	38.5 ±1.4	33.5 ±4.2
	1	26.7 ±4.2	28.1 ±3.4
	2	20.8 ±1.8	19.9 ±0.7
15	3	19.1 ±1.2	19.0 ±1.1
	4	19.3 ±1.2	19.3 ±1.5
	5	18.8 ±1.7	20.9 ±1.6
	6	19.6 ±1.3	23.4 ±1.5
20	7	20.2 ±1.2	24.8 ±1.3
	8	20.5 ±1.2	25.7 ±2.3
	9	20.9 ±1.2	25.7 ±3.3
	10	21.5 ±1.4	26.1 ±4.3
25	¹ Baseline values taken at -1 min. Proventil (free base albuterol, 89 µg/actuation, the equivalent of 108 µg albuterol sulfate) and liposomal metered dose albuterol sulfate (free base albuterol, 32 µg/actuation) aerosols administered at 0 minutes.		

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While the invention has been described with
reference to specific methods and embodiments, it will be
35 appreciated that various modifications and changes may be
made without departing from the invention.

IT IS CLAIMED:

1. A process for direct spray-drying a drug/lipid powder composition, comprising:
 - 5 (i) preparing an aqueous solution containing a water-soluble drug, said solution having no phosphate buffer salts, and a lipid-containing ethanol solution;
 - (ii) mixing the aqueous and ethanol solutions in a w/w ratio in the range of about 1:1 to 1:5, aqueous to
 - 10 ethanol, to form a solution mixture, said mixture having between about 3% to 4% (w/v) total solids;
 - (iii) spray drying the mixture and collecting the resulting dried particle powder.
- 15 2. The process of claim 1, wherein the water-soluble drug is selected from the group consisting of albuteral sulfate, ephedrine sulfate, ephedrine bitartrate, isoetharine hydrochloride, isoetharine mesylate, isoproteranol hydrochloride, isoproteranol
- 20 sulfate, metaproteranol sulfate, terbutaline sulfate, procaterol, and bitolterol mesylate, atropine methyl nitrate, cromolyn sodium, propranolol, fluoroisolid, ibuprofen, gentamycin, tobermycin, pentamidine, penicillin, theophylline, bleomycin, etoposide,
- 25 captopril, n-acetyl cysteine, verapamil, calcitonin, atriopentin, α -1 antitrypsin, interferon, oxytocin, vasopressin, insulin, interleukin-2, superoxide dismutase, tissue plasminogen activator, plasma factor 8, epidermal growth factor, tumor necrosis factor, granulocyte/macrophage colony stimulating factor, granulocyte
- 30 colony stimulating factor, macrophage colony stimulating factor, lung surfactant protein, and lipocortin.
3. The process of claim 2, wherein the water-
- 35 soluble drug is albuteral sulfate.

4. The process of claim 1, wherein the lipid-containing ethanol solution contains atleast one lipid selected from the group consisting of the following
5 lipids, their analogues and derivatives: phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidic acid, phosphatidylethanolamine, phosphatidylserine, sphingomyeline, and cholesterol.
- 10 5. The process of claim 1, wherein the lipid-containing ethanol solution contains phosphotidylcholine, phosphatidylglycerol, and .alpha.-tocopherol in a w/w ratio of about 167:15:0.1.
- 15 6. The process of claim 1, wherein said ethanol solution also contains CCl₃F and cholesterol.
7. The process of claim 1, which includes further drying the dried particle powder under vaccum.
20
8. The process of claim 1, wherein the dried mixture is rehydrated by the addition of an aqueous solution.
- 25 9. The process of claim 1, wherein said collecting further includes the packaging of the drug/lipid powder composition into packets, each packet containing a selected dose of the drug.
- 30 10. A method for administering a water-soluble drug to the respiratory tract at a selected dose, comprising, producing an airborne suspension a drug/lipid powder composition, containing such selected dose of drug, said drug/lipid powder composition formed by the steps of
35 (i) preparing an aqueous solution containing a water-soluble drug, said solution having no phosphate buffer salts, and a lipid-

containing ethanol solution,

(ii) mixing the aqueous and ethanol solutions in a w/w ratio in the range of about 1:1 to 1:5, aqueous to ethanol, to form a solution mixture, said mixture having between about 3% to 4% (w/v) total solids,

(iii) spray drying the mixture and collecting the resulting dried powder particles.

11. The method of claim 10, wherein the drug/lipid composition powder particles are suspended in a fluorocarbon propellant which does not solubilize the particles, and said producing includes storing the suspension under pressure in a cannister, and releasing a selected amount of the suspension from the cannister in an aerosolized form.

12. The method of claim 10, claim 10, wherein the drug/lipid powder particles are provided in moisture-free packets, each containing a metered dose of the drug in the powder, and said producing includes expelling the powder from the packet in an air-borne form.

13. The method of claim 12, wherein said producing is achieved using an airstream produced by user inhalation to entrain the drug/lipid powder particles and draw them into the user's respiratory tract.

14. The method of claim 12, wherein said producing is achieved using a spray device containing pressurized fluorocarbon propellant, a valve for releasing the pressurized propellant in an aerosolized stream, and means connecting the valve to the powder packet, effective to bring the aerosolized propellant stream into contact with the powder in the packet, to entrain the powder in the propellant stream.

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15. The method of claim 10, wherein the water-soluble drug is selected from the group consisting of albuteral sulfate, ephedrine sulfate, ephedrine bitartrate, isoetharine hydrochloride, isoetharine mesylate, isoproteranol hydrochloride, isoproteranol sulfate, metaproteranol sulfate, terbutaline sulfate, procaterol, and bitolterol mesylate, atropine methyl nitrate, cromolyn sodium, propranalol, fluoroisolidide, ibuprofin, gentamycin, tobermycin, pentamidine, penicillin, theophylline, bleomycin, etoposide, captopril, n-acetyl cysteine, verapamil, calcitonin, atriopentin, α -1 antitrypsin, interferon, oxytocin, vasopressin, insulin, interleukin-2, superoxide dismutase, tissue plasminogen activator, plasma factor 8, epidermal growth factor, tumor necrosis factor, granulocyte/macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor, lung surfactant protein, and lipocortin.

20

16. An apparatus for administering a water-soluble drug, at a selected dose, via the respiratory tract comprising:

a drug/lipid powder composition formed by the steps
25 of

- (i) preparing an aqueous solution containing a water-soluble drug, said solution having no phosphate buffer salts, and a lipid-containing ethanol solution,
- 30 (ii) mixing the aqueous and ethanol solutions in a w/w ratio in the range of about 1:1 to 1:5, aqueous to ethanol, to form a solution mixture, said mixture having between about 3% to 4% (w/v) total solids,
- 35 (iii) spray drying the mixture and collecting the resulting dried powder particles; and,

a device for producing an airborne suspension of the drug/lipid powder containing such selected dose of drug.

17. The apparatus of claim 16, wherein the
5 drug/lipid powder is suspended in a fluorocarbon propellant which does not solubilize the drug/lipid powder particles, and said device includes a cannister containing the powder/propellant suspension in pressurized form, and a valve connected to the cannister
10 for delivering a selected volume of the suspension in aerosolized form.

18. The apparatus of claim 17, wherein the fluorocarbon propellant is CClF_2CF_3 .
15

19. The apparatus of claim 16, wherein the drug/lipid powder particles are provided in moisture-free packets, each containing a metered dose of the drug in the powder, and said device includes means for expelling
20 the powder from the packet in an air-borne form.

20. The apparatus of claim 19, wherein the device uses an airstream produced by user inhalation to entrain the drug/lipid powder particles and draw them into the
25 user's respiratory tract.

21. The apparatus of claim 19, wherein the device is a spray device containing pressurized fluorocarbon propellant, a valve for releasing the pressurized
30 propellant in an aerosolized stream, and means connecting the valve to a powder packet, effective to bring the aerosolized propellant stream into contact with the powder in the packet, to entrain the powder in the propellant stream.

35

22. The apparatus of claim 16, wherein the water-soluble drug is selected from the group consisting of albuteral sulfate, ephedrine sulfate, ephedrine bitartrate, isoetharine hydrochloride, isoetharine mesylate, isoproteranol hydrochloride, isoproteranol sulfate, metaproteranol sulfate, terbutaline sulfate, procaterol, and bitolterol mesylate, atropine methyl nitrate, cromolyn sodium, propranolol, fluoroisolid, ibuprofen, gentamycin, tobermycin, pentamidine, penicillin, theophylline, bleomycin, etoposide, captopril, n-acetyl cysteine, verapamil, calcitonin, atriopentin, α -1 antitrypsin, interferon, oxytocin, vasopressin, insulin, interleukin-2, superoxide dismutase, tissue plasminogen activator, plasma factor 8, epidermal growth factor, tumor necrosis factor, granulocyte/macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor, lung surfactant protein, and lipocortin.

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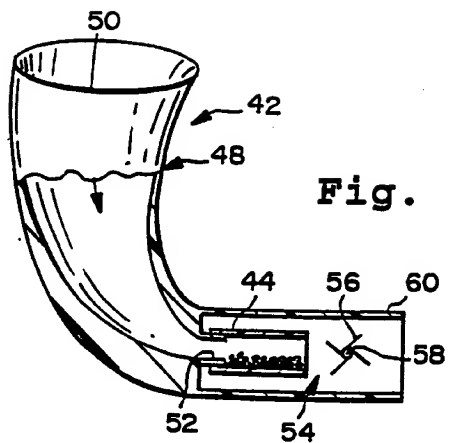
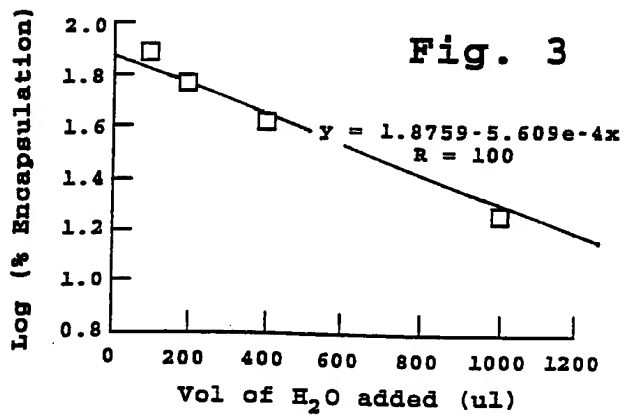
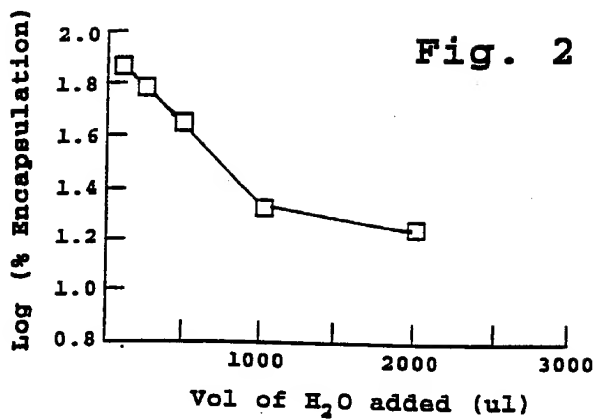


Fig. 1

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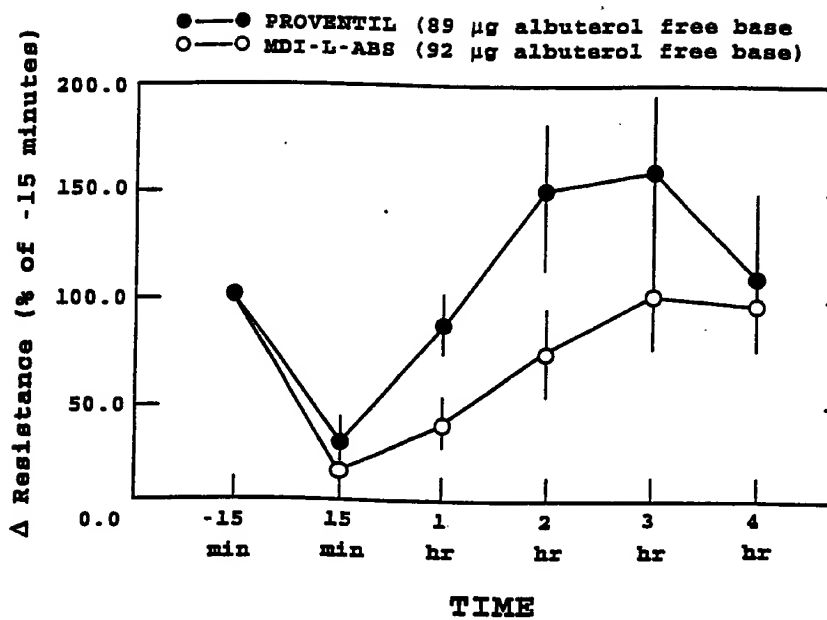
$\frac{3}{3}$ 

Fig. 4

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 91/03092

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl.5 A 61 K 9/14 A 61 K 9/127 A 61 K 9/72		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl.5	A 61 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	US-A-4 830 858 (PAYNE et al.) 16 May 1989, see the whole document; in particular examples 1,2	1,4
Y	---	2,3,5-22
Y	EP-A-0 260 241 (AKTIEBOLAGET DRACO) 16 March 1988, see the whole document; in particular page 8, example 17,; claim 15	2,3,5-22
A	WO-A-8 604 233 (RIKER LABORATORIES INC.) 31 July 1986, see page 3, lines 19-25	6
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents : ¹⁰</p> <p>^{"A"} document defining the general state of the art which is not considered to be of particular relevance</p> <p>^{"E"} earlier document but published on or after the international filing date</p> <p>^{"L"} document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>^{"O"} document referring to an oral disclosure, use, exhibition or other means</p> <p>^{"P"} document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>^{"T"} later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>^{"X"} document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>^{"Y"} document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>^{"A"} document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
27-08-1991	19. 09. 91	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	<div style="display: inline-block; border: 1px solid black; padding: 2px 10px;">M. PEIS</div> M. Pa3	